EFFECTS OF CONTINUOUS LOW INTENSITY RADIATION ON SUCCESSIVE GENERATIONS OF THE ALBINO RAT

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AN understanding of the consequences of low intensity environmental radiation during ontogeny, growth, and maturation of the mammalian species is of concern in evaluation of the effects of radiation on the hereditary fitness of populations. This paper deals with the effect of low intensity gamma radiation applied during the embryological periods, and in some studies the radiation was continued throughout the lifetime of the individual and for successive generations. The effect of the continuous radiation on successive generations of the albino rat used in these experiments was limited to 2r daily of continuous gamma radiation.

The effect of chronic gamma radiation on the growth, survival, life shortening, histology, pathology, and the hematology of the laboratory mammals have been the subject of numerous studies. The influence of continuous radiation on the reproductive system has been studied by some investigators, while the effect of the ionizing radiation applied during the prenatal periods on the functional integrity of the germ plasm as judged by the reproductive performance capability has been of considerable interest to the workers in this laboratory.

In considering the response of mammalian tissue to continuous radiation, LAMERTON (1959) concluded that it is very dangerous, in the assessment of the effects of continuous radiation, to extrapolate from data obtained with acute radiation experiments. He points out that under continuous radiation the response of tissues of the body may be essentially different from that under acute radiation. While it is reasonable to believe that all of the tissues of the body have some capacity for maintenance of the cellular population under continuous radiation, they will vary considerably in the time required to establish homeostasis and in the dose rates tolerated. Investigations of LAMERTON, STEEL, and WIMBER (1961) demonstrated that while the testes and splenic white pulp showed progressive cellular depopulation, the thymus and the bone marrow showed little further damage after three weeks of continuous whole-body radiation.

SACHER (1955) in comparing the effects of continuous radiation on a number of species of animals found that many or all physiologic functions are affected by radiation and that the lethal process is a weighted sum of the constituent systems. The investigations of STADLER and GOWEN (1964) have shown that mice may be maintained in a radiation field of 2.2r daily for over ten generations without deleterious effects. The litter size, the sex ratio, and the absence of visible point mutations, are all of direct bearing on this investigation.

Description of the Facility: The investigations reviewed in this article have been carried out in two radiation facilities located at Texas A&M University. The first described by KRISE, ROBINSON, and BROWN (1961) consists of a radiation chamber containing two central Co^{60} sources of one curie each, around which tiers of cages are arranged in concentric circles. The use of two radiation sources allows for a vertical arrangement of cages. In this small facility daily doses of 2, 5, 10, and 20r over a 23 hour period may be delivered to the cages. The control animals are maintained in a connecting area and are shielded by a thick concrete wall.

The second facility is much larger, containing 1250 curies of Co^{60} which makes possible doses from 1 to 200r daily. The animals are maintained in small portable airconditioned houses which may be positioned in the radiation field at the required dose level. The daily period of chronic low level radiation in this facility is 20 hours. In both of the installations the radiation levels were determined through the use of Victoreen-type dosimeters. All of these systems give a fairly consistent dosimetry.

Experiments

Effects of low intensity gamma radiation given during the entire prenatal period: A series of investigations have been carried out on the effect of continuous low intensity gamma radiation on the developing fetus of the albino mouse and rat. In these studies the animals were bred and placed in the chamber at the initiation of pregnancy. The young were removed at parturition and observed for any changes that might have been occasioned by this treatment. In a recently completed study of this type, COPPENGER (1964) has shown that 50r daily for a 20 hour period to a total dose of 1000r is not incompatible with the production of living offspring; however, these progeny exhibited abnormalities which consisted of a generalized reduction in body weight, microencephaly, and reduction in the weights of the liver, kidney, and spleen. Micropthalmia and anopthalmia were observed in 6.8 percent of the newborn. The livers of these animals show extensive hematopoietic islands of tissue. While irradiation at the level of 50r daily did not affect the total number of implantations, it did increase embryonic mortality in that 43 percent resorptions occurred as compared to 15 percent of the controls. The gestation period was increased by one day and parturition was long and complicated. The sex ratio of the newborn did not favor either sex; however, there was a preponderance of males in the 20-day irradiated fetuses. The survival of these irradiated animals was not altered during 350 postnatal days. The growth in weight of the males was retarded during 168 days; on the other hand, the females showed an increased weight gain. All animals were sterile and the gonads vestigial. In the males, the steroid hormones were active in that prostates of the males were similar in weight to those of the controls. No aberrant behavior was observed in the caged animals, however, a generalized reduction of cerebral size was observed.

The effect of low intensity chronic radiation on the production of successive litters of albino rats has been reported by BROWN, KRISE, and PACE (1963). After conception, groups of ten female rats were placed in the radiation chamber at the levels of 0, 2, 5, 10, and 20r daily and maintained continuously therein for their reproductive life. The successive litters showed no differences in size or in the number of offspring produced in the first four litters and there was no significant variation in the first six litters born at the 0, 2, 5, and 10r daily radiation levels. No anomalies were observed at any level of radiation employed in these experiments other than a slight decrease in the weight of the newborn at the 20r level. Offspring from the third litter born to mothers receiving continuous radiation of 0, 2, and 5r daily were shown to be fertile while those at the 10 and 20r daily were sterile. The weight gains in the animals receiving gamma radiation during prenatal life and maintained thereafter at control background was not significantly altered.

In a study on the effect of low intensity gamma radiation given to Swiss albino mice, CHANG, BROWN, KRISE, and SANDERS (1963) found that the combination of pre- and postnatal radiation produced sterility in the female at much lower doses than the male. Female mice receiving 10r daily for six weeks were sterile while the males were not sterile until 17 weeks of postnatal irradiation.

Effect of continuous irradiation during pre- and early postnatal life on reproduction in the rat: In this study Holtzman rats were exposed to continuous Co^{60} irradiation at rates of 1, 2, 5, 10, 20, and 40r per 23 hour day for 30 days beginning on the 15th day of gestation for total doses of 30, 60, 150, 300, 600, and 1200r respectively. There were no apparent significant effects from the irradiation on the dams which produced offspring at these treatment levels, at least with respect to the number of females littering and average litter size produced.

Total doses of from 300 to 1200r delivered at rates of 10, 20, and 40r for the 30 days affected the ability of the offspring to survive, as there was a significant decrease in the percentage of survivors at 23 days of age directly proportional to the total dose. Birth weights were significantly depressed ($P < .05$) in groups receiving 20 and 40r per day as compared with nonirradiated controls. The significant depression in weight at birth, 14 and 23 days of age in the group receiving 40r per day (1200r total dose) can be attributed to radiation damage since this depression persisted to the 300th day of life.

At 65 days of age, a high percentage of the animals receiving radiation at rates of 1, 2, and 5r per 23 hour day were, or would have been, fertile. Sires which had received 10, 20, and 40r per day did not produce litters when mated with known fertile females. Histological examination of the testes of the 10, 20, and 40r groups showed only a few tubules containing active germinal epithelium. At 170 days of age, the fertility data were essentially the same as that obtained at 65 days, with the exception of one male in the 10r group which sired a litter.

The data obtained on fertility, vaginal plug, mating activity and histological observations lead to the conclusion that a total dose of 300, 600, or 1200r at the rate of 10, 20, and 40r per day during late prenatal and early postnatal life produced complete sterility in male albino rats, accepting the one exception at the 300r dose level.

The average testicular weights at 65, 170, and 300 days of age were significantly depressed ($P < .01$) in the groups receiving 5, 10, 20, and 40r per day when compared to nonirradiated controls.

The effects of 5r (150r total dose) per day for 30 days on the developing offspring including the sensitive prenatal period is not as detrimental to the testis as 150r given acutely on days 17, 18, 19, 20, 21, or 22 of prenatal life. Rats receiving 150r at the rate of 5r per day were able to recover by 170 days of age, whereas, any group of offspring receiving 150r acutely on the prenatal days mentioned above were sterile except those irradiated on the 17th day of prenatal life in which only 66 percent were sterile (MURPHREE and PACE 1960).

There was a significant decrease in total number of tubules and average number of active tubules ($P < .05$) in the groups receiving 5, 10, 20, and 40r per day as compared with controls at 170 and 300 days of age. The size of the active tubules was decreased in the groups receiving 10 and 20r per day as compared with controls at 170 and 300 days of age. A complete absence of germinal epithelium was noted in the 40r per day group at 65, 170, and 300 days of age.

Therefore, it may be concluded, within the limitations of this experiment, that gamma irradiation delivered at rates of 10, 20, and 40r per day will cause permanent sterility in males and probably in females exposed from the 15th day of gestation through the 23rd day of postnatal life.

Effect of continuous gamma radiation on successive generations of the albino rat: An investigation was originally undertaken to ascertain the effect of low intensity radiation on fertility in the albino rat maintained in the radiation environment from the time of conception until they reached sexual maturity. In this study (BROWN, KRISE, PACE, and DEBOER, 1964) ten female rats of the Holtzman strain were placed in the radiation environment of 0, 2, 5, 10, and 20r daily at the time of conception. The young born to these females were maintained in the same environment until maturity at 134 days at which time they were bred to males having the same radiation history. Except for the group receiving 2r daily, a total pre- and postnatal radiation period of 134 days resulted in no litters from the irradiated pairs. In order to ascertain whether the males or the females were sterile they were bred to unirradiated control animals. In this case the males were sterile and the females fertile. As has been shown in this and numerous other studies, the testes of the male rats are much more sensitive to low intensity continuous radiation than the ovaries of the females. These observations are in contradiction to those made on the mouse in which the female gonads are more sensitive to both continuous and acute irradiation.

Since the progeny of the females of the Holtzman strain of rats placed in the radiation environment at 2r daily were all fertile when bred to mates having the same radiation history and the litter sizes resulting from these matings were within normal range, it was decided to continue this study over a number of generations in order to determine whether or not a germ line could be maintained. This investigation has extended over a period of five years. The procedure was established that when the young born in the radiation chamber reach the weaning stage, five males and five females were chosen at random and were maintained together in the radiation chamber until sexual maturity had been reached and conception had occurred. The successive generations of albino rats of this experiment, therefore, were never out of the radiation environment for the duration of the experiment which was over five years. The control strain was maintained in the same environment but separated from the radiation source by a thick concrete wall which reduced the radiation levels to essentially a background dose. The second generation of rats in this experiment will be designated F_2, the third, F_3, etc. At the end of the F_4, it was decided to increase the number of breeding animals to 10 males and 10 females randomly selected from the different litters of the same generation. When the offspring from these matings were weaned, the irradiated mothers were returned to the breeding cage and crossed with irradiated males of the same generation. This procedure was repeated until four successive litters were obtained. When litters were born they were examined for the presence of externally visible anomalies, sexed, and raised to the weaning stage. Only individuals of the first litter were used to produce the successive generations of the colony. The successive litters are designated by a subscript letter; thus the second litter born in the fourth generation is designated as F_{4b}. Unfortunately, in the seventh or eighth generation the rats developed a viral induced lung infection which has proven difficult to control. Both the control and irradiated animals have been about equally severely infected.

TABLE 1

Data on the litters born to rats receiving 2r daily for ten generations

Irradiated	Number of females	Percent females littering	Total born alive	Average litter size	Average individual weight (g)	Average weaning weight (g)	Percent alive at 21 days
Generation 4							
$F_3 \times F_3 = F_{4a}$ (1st litter)	16	87	114	8.1	5.4	no data	no data
F_{4b} (2nd litter)	10	100	81	8.1	no data	56.3	75
F_{4c} (3rd litter)	10	60	53	9.0	7.2	41.7	83
F_{4d} (4th litter)	10	50	41	6.8	5.1	59.0	85
Generation 5							
$F_4 \times F_4 = F_{5a}$	10	60	57	9.5	none taken	50.9	84.2
F_{5b}	10	80	73	9.1	6.0	41.6	45
F_{5c}	10	80	71	8.9	5.4	56.0	76
F_{5d}	10	40	30	7.5	8.3	87.0	70
Generation 6							
$F_5 \times F_5 = F_{6a}$	12	83	75	7.5	6.2	58.4	87
F_{6b}	12	75	74	8.2	6.0	81.4	73
F_{6c}	12	41	35	7.0	5.8	...	..
F_{6d}	12	16	9	4.5	8.3	...	..
Generation 7							
$F_6 \times F_6 = F_{7a}$	15	100	102	7.6	6.5	53.4	64
F_{7b}	10	100	67	6.7	6.4	39.8	100
F_{7c}	10	70	54	8.0	6.6	44.0	100
F_{7d}	in progress						
Generation 8							
$F_7 \times F_7 = F_{8a}$	10	70	55	7.8	6.6	44.0	98.1
F_{8b}	10	20	17	8.5	6.3	39.1	100.0
Generation 9							
$F_8 \times F_8 = F_{9a}$	10	50	38	7.6	5.8	42.7	65.8
$F_9 \times F_9 = F_{10a}$	in progress						
Generation 10							
$F_9 \times F_9 = F_{10a}$	20	30	37	7.5	5.9	38.2	73.3
F_{10b}	20	20	15	4.5	6.5	no data	no data
Generation 11							
$F_{10} \times F_{10} = F_{11a}$	23	82.6	118	6.8	6.4	51.7	66.1
F_{11b}	20	65.0	93	7.2	6.8	53.5	93.5
F_{11c}	20	40.0	67	6.9	5.2	50.2	92.8
F_{11d}	20	35.0	44	6.3	6.3	50.0	97.7
F_{11e}	20	20	5	1.25	7.0	55.0	100
Generation 12							
$F_{11} \times F_{11} = F_{12a}$	55	85.4	291	7.0	6.2	44.3	75
F_{12b}	21	95.2	154	5.7	6.5	46.2	83.4
F_{12c}	21	66.7	75	5.4	6.3	58.4*	83.7*

* At this time only nine litters with littering continuing.

TABLE 1—(continued)

Data on the litters born to rats receiving 2r daily for ten generations

Irradiated	Number of females	Percent females littering	Total born alive	Average litter size	Average individual weight (g)	Average weaning weight (g)	Percent alive at 21 days
Generation 13							
$F_{12} \times F_{12} = F_{13}$	30	80†	14.7	6.1	5.9	56	99
Controls							
Generation							
F_5	13	100	103	8.2	6.5	50.9	58.3
F_6	10	100	91	9.1	6.8	38.9	79.0
F_7	10	100	88	8.8	7.2	36.0	89.0
F_8	10	80	72	9.0	6.6	36.3	40.3
F_9	11	100	116	10.5	6.5	37.5	31.0
F_{10a}	10	100	85	8.5	5.9	...	...
F_{10b}	24	91.7	151	7.3	6.0	40.0	53.6
F_{11a}	24	87.0	183	9.6	6.4	47.9	69.9
F_{11b}	24	66.7	145	9.1	6.5	50.7	87.6
F_{11c}	24	50.0	81	7.7	7.4	53.4	85.2
F_{11d}	24	41.7	76	8.1	6.3	58.8	86.8
F_{11e}	24	25.0	44	7.5	6.2	51.4	84.1
F_{11f}	24	4.2	5	5	6.2	no data	no data
F_{12a}	47	100	467	10.0	6.3	52.6	72.6
F_{12b}	19	100	179	9.4	6.2	53.4	93.8
F_{12c}	19	84.0	179	11.2	6.4	50.4	93.0
F_{12d}	10	90.0	77	7.7	7.1	no data	no data
F_{13}	30	90.0‡	216	9.4	6.4	51.0	97.0

† Breeding in progress, incomplete data.
‡ 90 percent females pregnant with only 83 percent littering to date.

The data for the eighth and ninth generations, Table 1, is somewhat fragmentary owing to the endemic infection. However, in the ninth and tenth generations, much more uniform data were obtained. In the 11th generation an extensive testing program was undertaken to ascertain whether the previous radiation treatment had modified the heredity of the animals or whether the observed decrease in litter size was due to the direct somatic effects of the radiation on the developing embryos. In this test, 97 females of the 11th irradiated generation were bred to males of the same group in the radiation chamber and at conception 42 of these were transferred to the control chamber and 55 of the females were allowed to remain in the radiation environment. In a similar manner, 89 control females were bred to control males and 41 of these animals were transferred to the radiation chamber while 47 remained in the original control environment. The results may be seen in Table 2. In all cases the sex ratios were equal. The mean litter sizes of the two latter groups having a radiation history of 2r for 10 or 11 generations show a significantly decreased litter size of 7.8 and 7.0 (average 7.4) as compared to the control animals both in and out of the radiation chambers whose litter size were 10.5 and 10.4. The results of this experiment may be interpreted to mean that the radiation levels of 2r did not reduce the litter size in one generation, however, 2r given continuously for 11 generations had a cumulative hereditary effect resulting in a reduced number of individuals per litter.

The four groups of offspring obtained in the experiment just described were mated with control males or control females and some of the offspring were mated with others of the same radiation history. The matings are shown in Table 3 wherein the controls maintained in the control environment (C-C), the controls changed to the radiation environment (C-R), the offspring produced from irradiated parents in the control environment (R-C), and the irradiated animals maintained continuously in the radiation chamber (R-R) were crossed with control (C-C) males. The results are shown in the first two columns of Table 3. Except for the cross R-C female × C-C male the resulting litter sizes when the irradiated animals are crossed with the controls are within the normal range. The results in question from the cross, R-C female × C-C male, were based only on 13 litters, hence it is possible that chance variation accounted for this difference. When the animals of each group were bred to males of the same group (right most column in Table 4), the two groups having a history of no radiation C-C male × C-C female or of only one generation of radiation at 2r daily C-R male × C-R female had litters of normal size. However, when the animals having a history of many generations of low intensity radiation R-C female × R-C male and R-R female × R-R male, the litter sizes showed a significant decrease. No significant differences could be ascertained in the sex ratios of offspring born in this experiment. These data, therefore, suggest that hereditary factors resulting in a decreased litter size have been accumulated during the generations of continuous exposure. These factors are not demonstrable in the heterozygous outcross; however, they are much more likely to be manifested in the homozygous crosses. Since the litters were saved to weaning, no counts were made of corpora lutea, implantation sites, or resorbed embryos.

Since radiation has been shown to affect the morphology, the motility and the total number of sperm ejaculated by the male animal, a complete seminal analysis was carried out on the males of the 12th generation. The males tested were from the irradiated parents maintained continuously in a radiation background

TABLE 2

Data on reproduction in the F_{11} generation in and out of the radiation chamber

	C–C	C–R	R–C	R–R
Number of females in group	47	41	42	55
Percent females littering	100%	100%	81.0%	85.4%
Average litter size	10.4	10.5	7.8**	7.0**
Number of female offspring	247	181	119	144
Number of male offspring	222	193	114	148
Percent alive at 23 days	73%	87%	81%	75%

C–C = Control females maintained in the control environment continuously; C–R = Control females transferred to radiation chamber at the time of conception; R–C = Irradiated females transferred to control environment at time of conception; R–R = Irradiated females maintained continuously in radiation environment for 11 generations.
** Highly significant by analysis of variance and Duncan's multiple range test.

TABLE 3

Data on reproduction in different groups of rats receiving the treatment shown in Table 5 ($F_{12} \times F_{12} = F_{13}$) when crossed with control mates and when crossed with others of the same group

	CC ♀	CC ♂	Homozygous cross
CC	35 9.5 151♂ ♀159	35 9.5 151 159	35 9.5 151 159
CR	24 9.3 83 121	25 11.4 139 138	33 9.2 157 133
RC	15 10.9 71 83	13 7.8 45 62	18 7.1 51 62
RR	12 8.7 49 54	13 9.0 48 48	23 6.6 62 76

C–C = control females maintained in control chamber continuously; C–R = control females transferred to the radiation chamber at time of conception; R–C = irradiated females transferred to the control chamber at time of conception; R–R = irradiated females maintained continuously in the irradiation chamber. The number in the middle of each square represents the average litter size. The numeral in the upper right of each square represents the number of litters produced. The lower left figure is the total number of males, and the lower right figure in each square the total number of females.

TABLE 4

Results on the analysis of semen of albino rats having the following radiation history

	C–C	C–R	R–C	R–R
Rate of motility	2.4	2.1	2.4	2.2
Percentage motile	63	60	67	63
Percentage normal	88	86	89	88
Number headless tails	8	10	8	8
Number coiled tails	4	4	3	4
Total number sperm $\times 10^3$	796.5	517.7	749.7	835.3

C–C = Control animals having only background radiation; C–R = Control animals born to females placed in the radiation chamber on first day of pregnancy and receiving 2r daily for 11 generations; R–C = Animals born to females irradiated continuously at 2r daily for 11 generations and placed in the control chamber at the time of conception; R–R = Animals born to females remaining continuously in radiation chamber at 2r daily for 11 generations.

(R-R), or from irradiated parents transferred to the control room at 90 days of age (R-C). The other two groups were from control parents transferred to the radiation chamber at 90 days of age (C-R) or maintained continuously in the control environment (C-C). A technique which has been worked out in this laboratory for obtaining fluid semen samples (LAWSON and SORENSEN, in press) from albino rats was employed. This technique involves the removal of the coagulating gland by surgical excision followed by a recovery period, after which electroejaculation produced fluid semen. The analytical procedures applied to the semen samples were the standard methods used on larger animals. From the data shown in Table 4, it may be observed that no demonstrable differences existed in the various groups with respect to the total number of sperm per mm^3 of ejaculate. Neither the percentage motile sperm, the percentage of normal sperm or the presence of headless nor tailless sperm differ significantly in the samples tested.

Since it has been reported that irradiated male rats show decreased mating activity, observations were made on ten male siblings of each of the groups designated in the preceding paragraph. The timed mating activity on this limited group of animals observed on three successive occasions was not appreciably altered by the radiation treatment of any groups tested.

Behavioral observations on animals subjected to 12 generations of continuous radiation: Measurable behavioral changes may be noted following radiation as well as other treatments even in the absence of discernible anatomical change. This point, suggested by FURCHTGOTT (1963), seems especially relevant considering the Russian workers' frequent reports of altered conditioned responses following very small doses (e.g., 2r).

Several recent reviews (STAHL 1960; FURCHTGOTT 1956, 1963) have revealed a number of studies focused on the later behavioral effects of doses below 100r X rays delivered during the various stages of prenatal and early postnatal development. In these studies deficits in motor responses, motivation and conditioning have been reported. With respect to very low prolonged doses, a recent study by GILBERT and GRAHAM (1964) is especially noteworthy. These workers reported a motivational change in adult rats following 3r weekly for 13 weeks. Western investigators rarely report behavioral effects of such doses in the adult. The suggestion seems to follow that a comparable radiation regimen beginning at conception should yield even more profound effects.

With regard to the present investigation, the literature reveals no equivalent opportunity to assess the cumulative genetic and ontogenetic behavioral effects of prolonged, low level exposure.

The present series of tests was undertaken primarily as a pilot venture with the hope of uncovering promising leads for more intensity study. The animals employed were the male offspring of the female rats having a history of 11 generations of continuous low intensity radiation of 2r over a 23 hr period daily. The corresponding controls were maintained for approximately the same number of generations at background levels of radiation. At the time of conception some of the females with a radiation history were transferred to the control room while some of the controls were transferred to the radiation room. Therefore, four types of offspring were available for this study: controls (C-C), controls born in a radiation environment (C-R), irradiated animals born in a control environment (R-C), and irradiated animals maintained continuously in the radiation chamber for successive generations (R-R). Each group contains 15 adult male animals and each subject was given six tests; the first four in random sequence for the purpose of controlling order effects. The two most stressful tests were given last in order of increasing stressfulness, as indicated below.

1. *Swimming to exhaustion:* Subjects were placed individually in water tanks 18 inches deep and 23 inches in diameter and were removed following exhaustion. If a subject remained underwater for 12 successive seconds without reappearing, the criterion for exhausion was met. The water contained a small amount of detergent for purposes of diminishing surface tension and insuring that the subjects were thoroughly soaked. Table 5 presents the mean swimming times

TABLE 5

Behavioral tests

C–R	R–C	C–C	R–R
Mean swimming time (minutes) to exhaustion			
27.42	26.78	40.67	26.73
Mean time (minutes following onset of the heat lamp when subjects jumped into the pit)			
1.35	1.48	1.31	1.24
Mean angle of inclination (degrees) when subject lost his balance			
50.0	49.9	48.6	51.1
Number of subjects in each group showing a negative gravity response			
4	10	7	7

C–C = Control animals born in control environment; C–R = Control animals born in radiation environment; R–C = Irradiated animals born in control environment; R–R = Irradiated animals born in radiation environment.

prior to exhausion for the respective groups.. Analysis of variance revealed a significant difference between the groups ($F = 6.45$; $df = 3/56$; $P < .01$). The critical difference method indicated that the locus of significance was between group C-C and all others, i.e., group C-C swam significantly longer than all other groups. The swimming test was given last for all subjects following 24 hours of rest from the previous testing.

2. *Conflict resolution:* Subjects were placed individually on a ledge 7 × 4 inches which was situated at a height of 34 inches. At one end of the ledge was a 250w heat lamp and at the other a "visually bottomless pit" (a black box 22 × 28 × 34 in high). Time (minutes and hundredths) to jump into the pit following the onset of the lamp was recorded. Table 5 shows the mean times for the respective groups. No significant differences were found ($F = .45$; $df = 3/56$; $P > .05$). It was felt that this test might reveal indecisiveness on the part of irradiated subjects (previously reported by CASEY, 1963) which would be reflected in longer times before jumping. This test was given next to last for all subjects.

3. *Open field:* This test is generally considered to be a test of emotionality and general exploratory activity. The open field was 42 × 48 × 12 inches high and contained four concentric rectangles 6 inches apart. Individual trials were 8 minutes in length. Observations were carried out behind a one-way screen and consisted of the following: (a) the number of rectangles entered (b) the number of times each rectangle was entered (c) the time (seconds) spent in each rectangle, and (d) the incidence of urination and defecation. Scores yielded by behavior in the more central rectangles were highly variable and badly skewed toward the zero end of the scale. Therefore, appropriate nonparametric techniques were applied. No significant differences between the groups were found in any of the various analyses undertaken.

4. *Adjustment to an inclined plane:* Ability to perform the necessary postural adjustment to maintain balance on an inclined plane was tested as follows: Subjects were placed individually in a lucite box 5 × 12 × 5 inches high with an unpainted pressboard floor. An open end of the box projected over the same "visually bottomless pit" described above. The angle of the box was set at zero. At exact intervals (approximately 2 seconds as cued by a timing gear) the angle of tilt was increased in 3 degree increments until subject lost his balance and fell into the pit. The angle of inclination at this point was recorded. The means in degrees for the various groups appear in Table 5. Differences were nonsignificant ($F = .24$; $df = 3/56$; $P > .05$).

5. *Geotropic response:* Previous investigators (MUNN, 1950) agree that most rats will show a response negative to gravity when placed on an inclined plane. In the present study only 28 of the 60 subjects showed such a response. Subjects were placed individually in a starting chamber 6 inches from the bottom of a plane 13 × 29 inches. The floor of the plane was covered with canvas and tilted at 45 degrees. After ten seconds a subject was released and his movements recorded. A negative geotropic response was counted when the subject reached a flat platform at the top of the plane. Many subjects changed directions several times before proceeding to the

bottom of the plane. Table 5 indicates the number of subjects in the various groups showing the negative gravity response. Differences between these frequencies were found to be nonsignificant ($\chi^2 = 3.58$; df = 3; $P > .05$).

6. *Coordination:* Subjects were placed individually on an elevated walkway consisting of two strands of #10 galvanized wire spaced 2¼ inches apart. A floodlight was situated at the subject's back and a shaded area was placed at the opposite "goal" end. Normally, strong light functions as a negative incentive for albino rats. Unfortunately, the present task appeared to be too difficult. Most subjects "froze" during the 4 minute trials and failed to meet any of the criteria set for measuring coordinated movement. No formal analyses of the data were undertaken. Pilot work on suitable tests of coordination are being carried out at the present time in this laboratory.

Differences of a significant degree were found only in connection with the swimming test—a test of resistance to exhaustion. The mean swimming times for all groups with radiation involvement (R-R, R-C, C-R) were all quite similar and all were significantly inferior to group C-C. The interpretation of this finding is unclear. The inferior performance of group R-C would suggest a genetic basis while the inferior performance of group C-R an ontogenetic basis. However, if both bases were operative as in the case of group R-R some additive effects would normally be expected. Although group R-R was inferior to group C-C, its similarity to groups R-C and C-R indicates no such additivity of effects.

The remaining results tend to support the general trend of western behavioral research findings, namely that little or no behavioral effects follow low dosages.

Since the production of successive generations of rats for this study was carried out in a relatively uniform manner, the calculation of the dose of irradiation to the gametes of each generation may be postulated. Each animal was exposed to gamma irradiation from the Co^{60} source for 21 days of prenatal life, 21 days to weaning and an additional 42 days to attain breeding age. Thus each generation was exposed to 84 days of irradiation. The total dose per generation would then be approximately 164r (measured in air in the midline of the cages). Since each subsequent generation was produced by mating irradiated parents, the total gametic or genetic dose becomes a question of viewpoint. Table 6 presents two possibilities as total accumulated ancestral dose for the 10th, 11th, 12th, and 13th generations.

DISCUSSION

The events occurring in low intensity irradiation are not without effects on the germ line of the albino rat as indicated from the data obtained in these studies. Despite the statistical significance of these results, the margin of injury is so low as to suggest that experiments of this type should be replicated before far-reaching

TABLE 6

Total dose of gamma irradiation received by successive generations of albino rats irradiated at 2r per day

Generation	r	2r
F_{10}	1476	2952
F_{11}	1640	3280
F_{12}	1804	3608
F_{13}	1968	3936

conclusions may be drawn. An unexplained genetic drift might have accounted for the decreased litter size and perhaps decreased stamina or lifespan in the successive generations of animals. On the positive side one might be content with the fact that the repair process of the protoplasm is such that damage produced by the ionizing events are either eliminated or repaired to such an extent that the germ line is able to maintain itself in a radiation field far in excess of what is considered to be tolerable limits. From the standpoint of the individual, the investigation is not rewarding. Months of work, many dollars, and much effort has gone into a study only to find at the end of five years that the question of the extent of the damage, if any, is unsolved. On the other hand, it may be comforting in face of the increasing radiation background to know that this question exists, and was not solved in a clear and unequivocal manner by manifestations of large amounts of damage to the hereditary fitness of a single mammalian species. Certainly many more investigations must be carried out on many other species before it is possible to access the deleterious effects of low intensity continuous radiation.

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SUMMARY

Irradiation during the entire prenatal life of rats delivered at 50r per 20 hour day (1000r total dose) was not incompatible with production of living offspring. The progeny, however, exhibited a limited number of anomalies such as reduction in body weight, microencephaly and a reduction of weight of certain organs, i.e. liver, spleen, and kidney.

Irradiation at rates of 2, 5, 10, and 20r per day to mature females has been shown to be ineffective in preventing reproduction at least through four successive litters in the albino rat. However, offspring receiving 10 and 20r daily throughout prenatal and postnatal life were sterile. The male rat was shown to be much more sensitive than the female rat with regard to radiation-induced sterility.

In albino mice irradiated continuously from prenatal through various postnatal periods, the female proved to be the most susceptible when sterility was considered.

Exposure of albino rats from the 15th day of gestation through the 23rd day of postnatal life to rates of 1, 2, 5, 10, 20, and 40r resulted in dose-dependent damage to the testes. Dose rates of 10, 20, and 40r per day (total doses of 300, 600, and 1200r) were found to produce permanent sterility in the males.

Exposures of 2r per day given continuously for 11 generations appear to have had a cumulative hereditary effect resulting in reduced litter size.

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